p53 mutations In lung cancers from non-smoking atomic-bomb survivors

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Tobacco smoke contains many carcinogens and has been linked with the development of lung cancer. We sequenced the conserved regions of the p53 tumour suppressor gene in lung cancers from 17 non-smokers from Hiroshima, Japan; 9 were atomic-bomb survivors. The mutations were predominantly transitions (all G:C to A:T); there were no G:C to T:A transversions. By contrast, lung cancers from 77 Japanese smokers have a predominance of G:C to T:A transversions in which the guanine residues occur on the non-transcribed DNA strand. These findings further implicate tobacco smoke carcinogens in the molecular pathogenesis of lung cancer.

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p53 mutations are common in human lung cancer[1] and the frequency is related to lifetime cigarette consumption.[2] In tobacco-associated lung cancer[1] mutations are widely distributed throughout exons 4–9, the prevalent mutation is G:C to T:A transversion with a predominance of guanine residues on the non-transcribed DNA strand, and the frequency of transitions at CpG sites is lower than in most other cancers. Tobacco smoke contains a mixture of highly mutagenic polycyclic aromatic hydrocarbons (PAH), which preferentially attack guanine bases. Strauss[3] suggested that the predominant guanine to thymine transversions occur on DNA replication, either by mispairing of the PAH-adducted guanine with adenine or by preferential insertion of adenine opposite the non-instructive modified base (the A rule).[3] We undertook molecular epidemiological studies to seek genetic damage attributable to atomic-bomb radiation.

Cases were selected from a prospective epidemiological study of 100000 residents of Hiroshima, Japan. All individuals with lung cancer were found by computer and those who had never smoked were selected. Individuals with estimated lung tissue radiation doses above 0·1 Gy (estimates based on DS86) were matched with non-exposed, non-smoking controls for age at the time of bombing and at death (within 5 years) but not histological type of lung cancer. All but 1 (patient 14) of the subjects were female.

Patient (histology)	Age (yr) (At bombing/ at diagnosis)	Radiation exposure*	DNA base (codon)	Amino-acid change
Exposed				
1 (SQ)	54/69	1·75	..	..
2 (AD)	35/59	1·56	..	..
3 (AD)	54/80	0·79	GCC→CCC (159)	Ala→Pro
4 (AD)	38/64	0·78	..	..
5 (SQ)	38/58	0·39	CAT→TAT (179)	His→Tyr
6 (AD)	53/78	0·25	..	..
7 (AD)	52/68	0·22	..	..
8 (AD)	54/73	0·10	CCT→CAT (191)	Pro→His
9 (SQ)	54/75	0·07	CAT→TAT (193)	His→Tyr
Controls				
10 (AD)	58/69	..	ATG→AAG (246)	Met→Lys
11 (SQ)	56/75	..	CGC→TGC (156)	Arg→Cys
12 (AD)	54/76	..	..	..
13 (AD)	42/62	..	..	..
14 (LC)	50/78	..	..	..
15 (SM)	37/76	..	G→A (intron 5)	Splice site
16 (AD)	33/62	..	..	..
17 (SM)	41/62	..	CAG→TAG (167)	Gln→Stop

Histology: SQ= squamous cell carcinoma; AD=adenocarcinoma; LC=Large-cell carcinoma; SM=small-cell carcinoma.
*Lung tissue exposed dose (Gy), estimated from DS86.

Table: **p53 mutations in lung cancers from atomic-bomb survivors and controls**

P53 coding and splice-site sequences for exons 5–8 were analysed in tumour tissues.[4] Mutations were confirmed by a second round of polymerase chain reaction amplification and sequencing, and germline sequences were examined in the mutated exons by analysis of DNA from non-neoplastic tissues. For comparison we collected smoking histories for 85 reported Japanese cases of p53 mutations (77 smokers, 8 non-smokers).[2,5–8]

We found no differences in p53 mutational spectra between atomic-bomb survivors and the non-exposed non-smoking controls (table). When the mutational spectra of 8 non-smokers in this study plus 8 previously reported non-smokers were compared with those of the 77 reported smokers, there were differences in the frequencies of all transitions (69 *vs* 35%, $p < 0·05$), G:C to A:T transitions (69 *vs* 21%, $p < 0·001$), and G:C to T:A transversions with a DNA non-transcribed strand bias (0 *vs* 29%, $p < 0.01$, Fisher's exact test). The results did not change after adjustment for histological type, age, and sex.

This study was designed to identify genetic lesions characteristic of radiation damage among atomic-bomb survivors with lung cancer. However, the p53 mutational spectra did not differ significantly between radiation-exposed and non-exposed non-smoking patients. It is likely that atomic-bomb survivors carry characteristic genetic lesions, but it will be necessary to study more cases and to use methods for detecting large-deletion type mutations.

The overall frequency of mutations among our 17 non-smokers (8/17 [47%]) and the frequency by histological type were similar to those reported among smokers.[2,5] However, G:C to A:T transitions were more common and G:C to T:A transversions much less common than in smokers, as reported previously.[2,5–8] The predominance of transitions suggests that endogenous mechanisms such as DNA polymerase infidelity, deamination of 5-methylcytosine, and spontaneous depurination are the main mutational mechanisms contributing to lung cancer in non-smokers.

The frequency of G:C to T:A transversion on the non-transcribed DNA strand showed a significant ($p < 0·01$) relation with cigarette exposure (figure). These results are consistent with the model of preferential repair

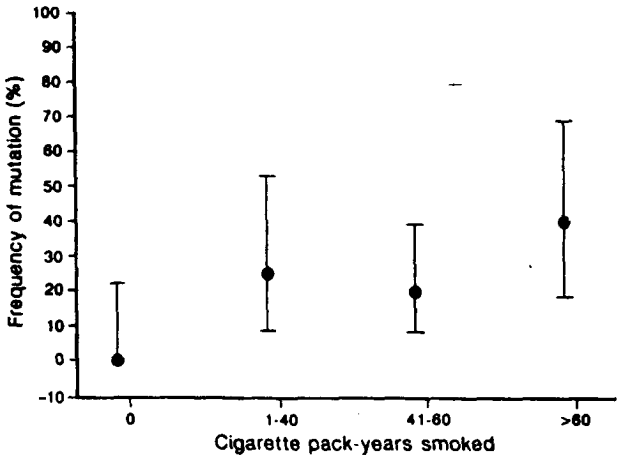

Figure: **Frequency of G:C–A:T transversions by smoking history**

of the transcribed DNA strand in actively transcribed genes.[9] Chemical carcinogens in tobacco smoke would be enzymically activated, and would preferentially bind to guanine residues to form DNA adducts in the bronchial epithelium. The strand bias would be generated by preferential repair of DNA adducts in the non-transcribed strand. Evans et al[10] have shown that the p53 gene is rapidly repaired and the transcribed DNA strand is preferentially repaired.

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